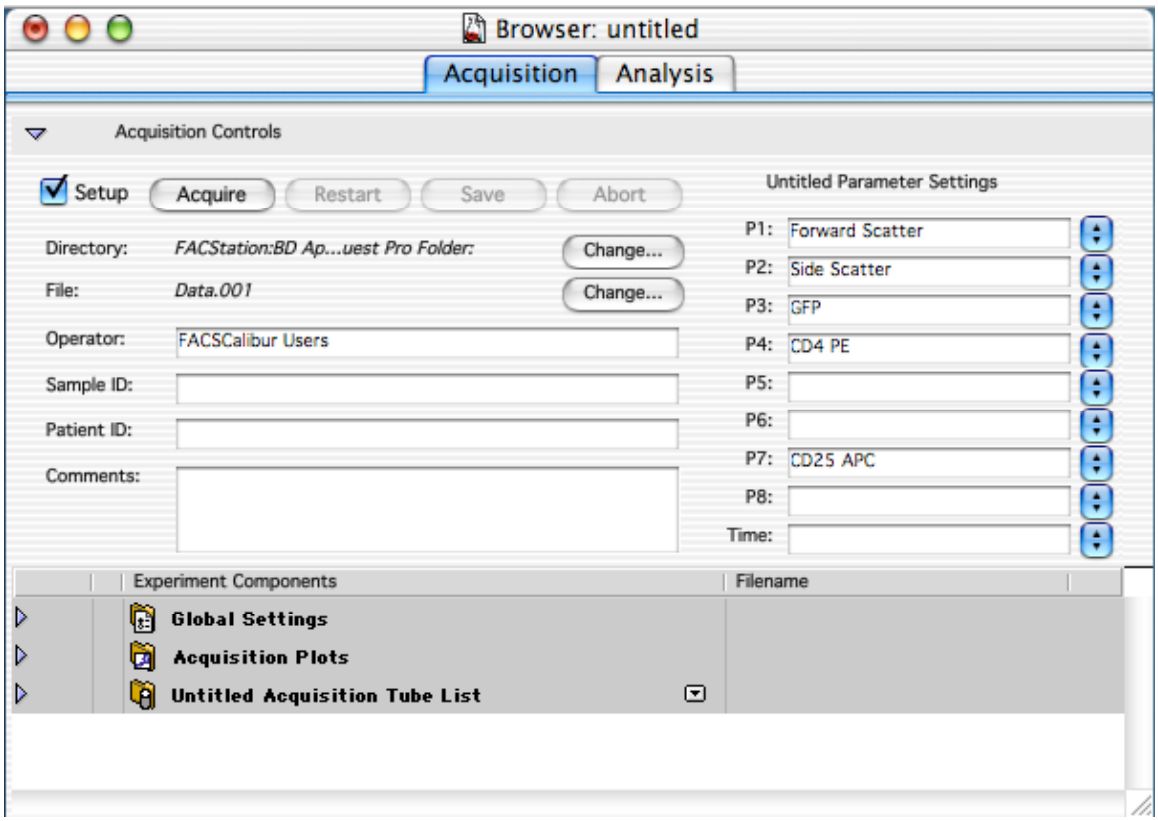
CellQuest Pro basics

This is not intended to be a substitute for the manual and does not take the place of training. Rather, it is simply to remind you of the basics. If you need additional information, refer to the FACSCalibur and CellQuest Pro software manuals. You can also contact Wolf at 301-435-7272 or lindwasw@mail.nih.gov. Many thanks to Jen Gillette for writing a draft of these protocols.

The Browser window:

After you open CellQuest Pro, the only open window will be a blank document. You will use this to monitor your sample acquisition and to perform data analysis. But before you can get started you'll need a bunch of other windows. Open the Browser window by going to the ACQUIRE pulldown menu and choosing "Connect to Cytometer". The Acquisition Control window will open as well. If you close the Browser by accident and you are already connected, you can open the Browser again by selecting ACQUIRE -> Browser. Note that when you begin you are in Setup mode.

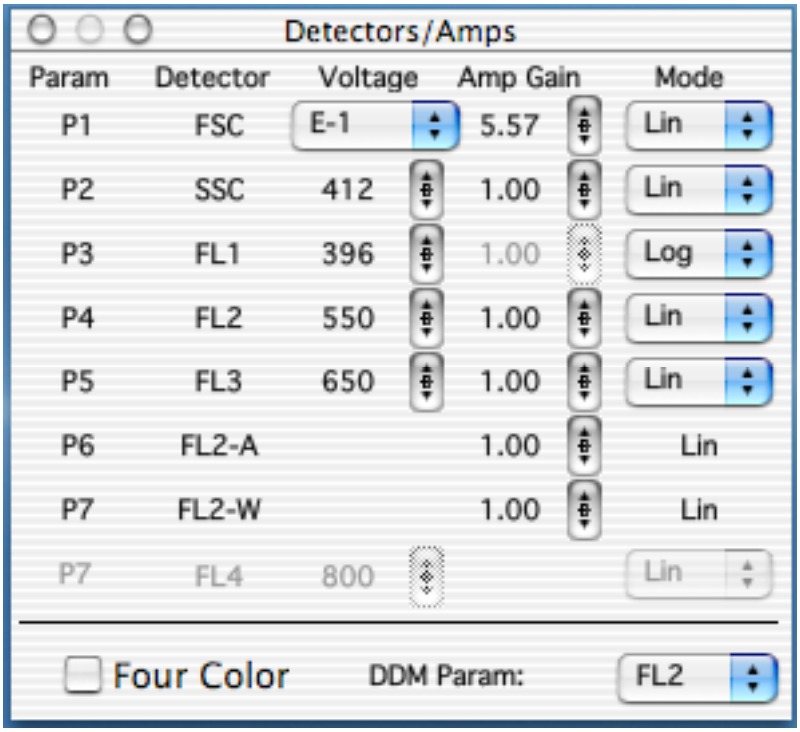


On the Browser window you will select the destination for you files (Directory: press Change and follow the prompts), a naming convention for your files (default is Data.001, .002, etc.), and which parameters you want recorded (you can name the parameters by choosing from the button menus on the right or type in your desired name).

Each sample reading generates a separate file. I prefer to name them myself by typing in the name each time under Sample ID. In order to get around the default file naming, press File: Change, delete the Custom Prefix, enter File prefix name –> Sample ID and Suffix –> None.

Parameters and Detectors/Amps:

There are seven different parameters (P1 - P7), each of which is linked to one of several detectors (PMTs or photomultiplier tubes). You can adjust the voltage on each PMT by opening the Detectors/Amps window (CYTOMETER -> Detectors/Amps). I will only discuss a few of the parameters here.



P1: FSC (Forward Scatter).

The FSC PMT measures light scatter from the 488nm laser after it passes through the sample. It is positioned in the beam path. FSC values are roughly proportional to cell diameter. Typically, we measure FSC in linear (Lin) mode.

P2: SSC (Side Scatter).

The SSC PMT also measures light scatter from the 488nm laser after it passes through the sample. It is positioned 90° from the beam path. SSC values are a measure of granulosity or cell shape. SSC is also typically measured in linear (Lin) mode.

P3: FL1

FL1 is used to detect green fluorophores such as FITC, AlexaFluor 488 or GFP. The 488nm laser excites the sample and passes through a filter on its way to the FL1 PMT. Typically, fluorescence (FL) is measured in log mode, but this will depend on the experiment.

P4: FL2

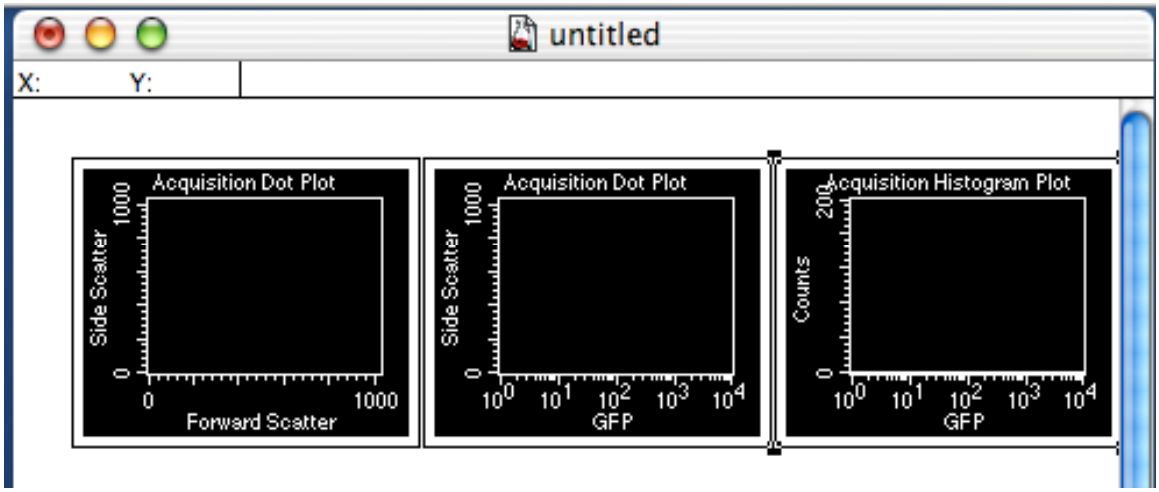
FL2 is used to detect PE. This fluorophore can be excited by the 488nm laser but has a much greater Stokes shift than FITC. FL2 will detect longer wavelength (yellow-orange) emission. Note that experiments using both FL1 and FL2 require Compensation, which will be covered in another protocol.

P7: FL4

This is the only parameter that uses the 635nm laser. To use it, check the Four Color option in the Detectors/Amps window. FL4 is used to detect far red fluorophores such as APC.

Plots and Setup:

Before you can adjust any of the instrument settings, you will need to open a bunch of plots in the document window. Here is an example of plots you will need for a one-color experiment.



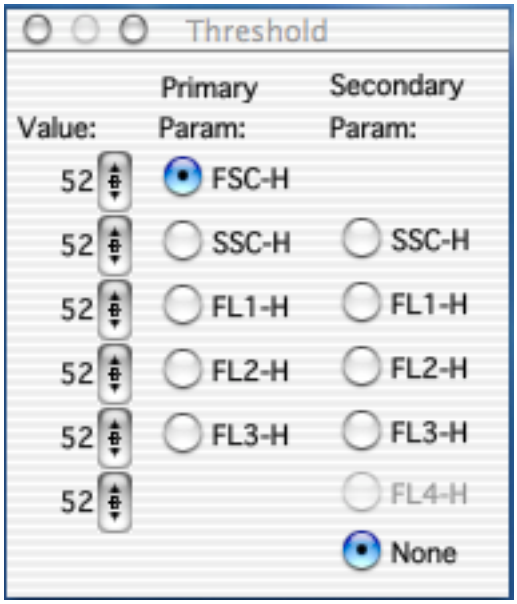
The first is a dot plot (PLOTS -> Dot Plot). When you open a plot, the Inspector window will open, allowing you to choose parameters for each axis, etc. You can choose any parameter that you have selected in the Browser window. For this plot select Plot Type -> Acq/Analysis on the Inspector. This plot will be used to monitor Forward and Side Scatter values for the cells in your samples.

The middle plot is also a dot plot and is used to monitor FL1 (in this case it's GFP fluorescence). It's also set to Acq/Analysis.

The last plot is a histogram (PLOTS -> Histogram) where you can see cumulative results from your sample. Again it's set for FL1. It's also set to Acq/Analysis.

Put one of your samples into the SIP. This should contain unlabeled cells. Press RUN on the cytometer and the ACQUIRE button on either the Browser window or the Acquisition Control window.

You should see dots appear on the dot plots. Each dot represents a cell. There will usually be a small amount of debris (very low FSC and SSC values), some dead cells and a lot of live cells.



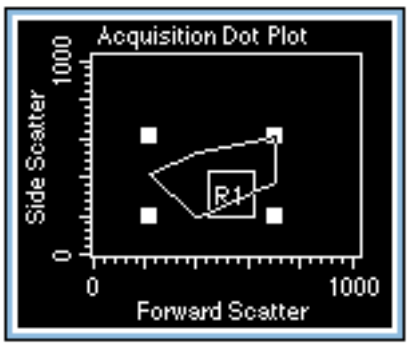
Most debris will fall below a default threshold FSC value of 52 and will not appear on the plot. You can open the Threshold window (CYTOMETER -> Threshold) to change its value, although that is rarely necessary.

The dead cells generally have lower FSC values than live cells. You want the live cells to be roughly in the middle of the FSC/SSC plot. You can adjust the position by changing the settings on the Detectors/Amps window.

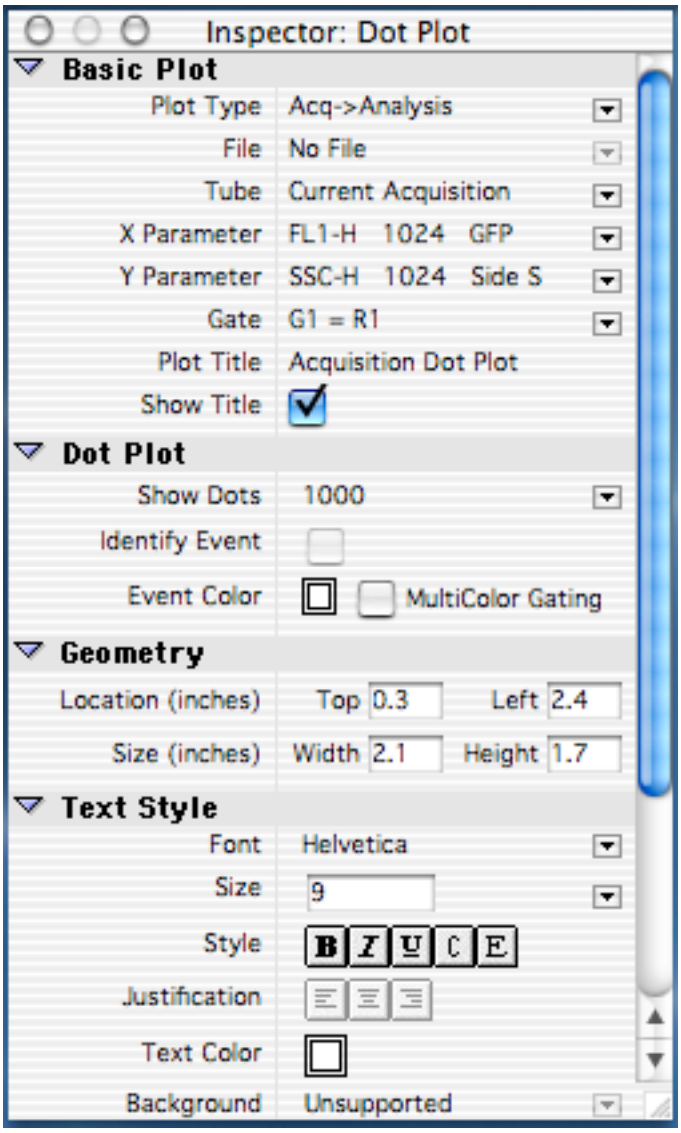
For example, the default settings for FSC (Voltage E0, Amp Gain 1.00) are perfect for lymphocytes. For larger cells (e.g. HeLa) you will need to lower the voltage to E-1 and increase the gain to about 5 or 6 to get the cells in the middle of the range. For SSC you usually only need to adjust the voltage.

Once you set FSC and SSC, you will usually want to gate around live cells. This is done using one of the region draw tools:

These are the region draw tools. I usually use the polygon.



Press PAUSE on Acquisition Control. Draw a region around the cells you want to measure (live cells). This region will be called R1.



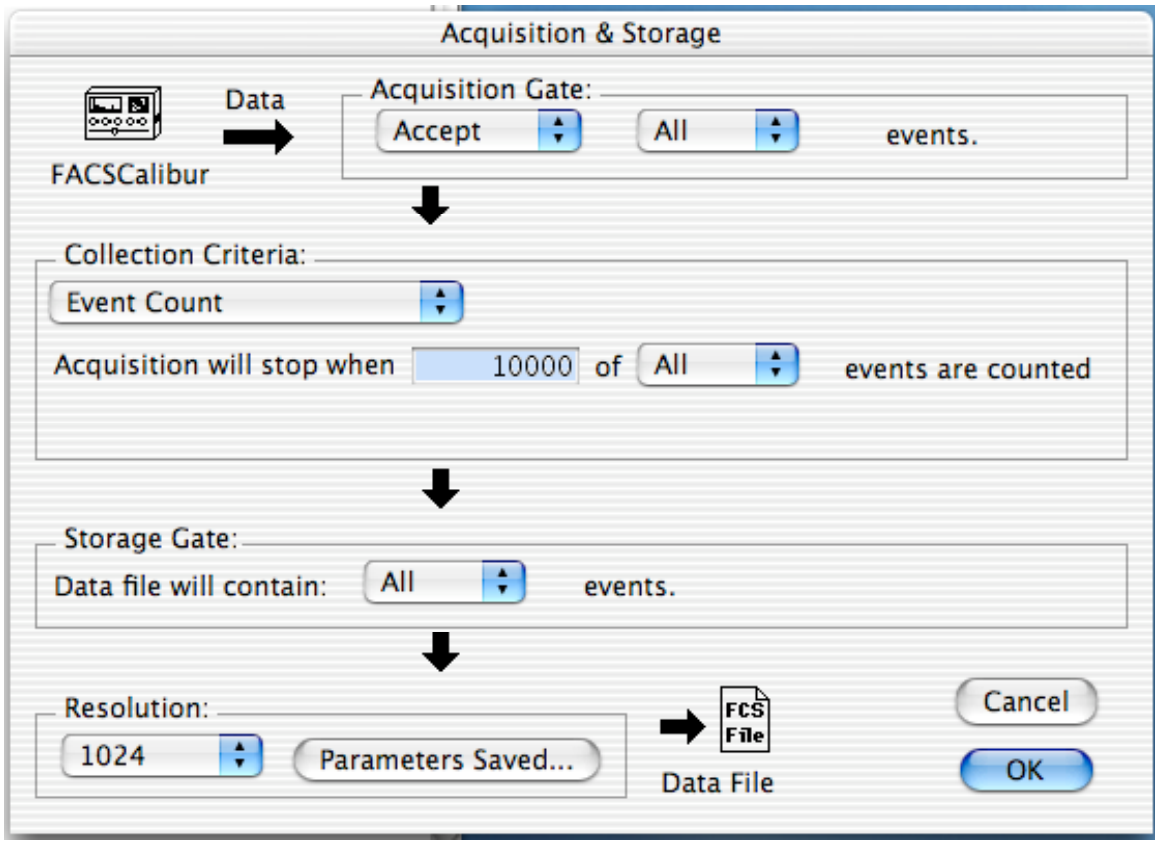
To "gate" that region, click on the GFP/SSC plot (the one in the middle) and choose Gate: G1 = R1 on the Inspector window. Press RESUME on Acquisition Control. You will be looking at FL1/SSC values for the cells that pass through the gate drawn on the FSC/SSC plot.

Remember that your sample is *not* labeled, so the only fluorescence should be autofluorescence. Adjust the FL1 setting (if it's in log mode you can only adjust voltage) until the cells are all in the first log. Values in this range are considered negative for fluorescence. At this point your Setup is finished. Put the cytometer back on STANDBY.

Sample acquisition:

To exit Setup mode, press PAUSE and then ABORT on Acquisition Control. Then uncheck Setup. You will now need to tell the machine how many cells you want it to count. A standard experiment will measure 10,000 cells. You can go as low as 3000 if necessary but your data will look lousy. There more cells you count, there more

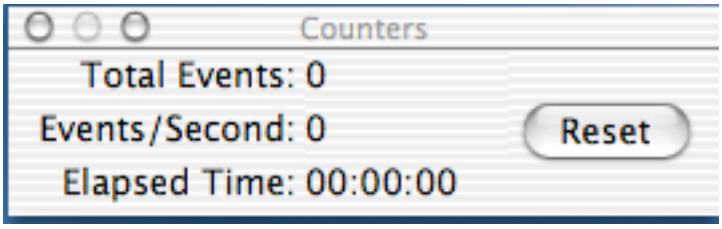
robust and pretty your data will be. Go to the ACQUIRE menu and choose "Acquisition & Storage".



For Collection Criteria: type in the number of events (cells) you want counted if other than 10,000. If you have gate, you can have the acquisition stop when the selected number of "G1=R1" (or another gate if you have one) events are counted instead of "All" events. This means that it won't stop measuring cells until you have 10,000 of the ones you want.

For Storage Gate: you usually want your file to contain "All" events. This way you can look back at the ungated cells when you do your analysis.

You are now ready to collect data. Give your sample a name by either typing it in Sample ID on the Browser window (you can call it "unlabeled" or "control") or using one of the presets (Data.001, etc.). Press RUN on the cytometer and ACQUIRE on Acquisition Control. The cytometer will count until it reaches 10,000 cells and them automatically save the data as a separate file in your pre-selected Directory. You can keep track of progress by opening the Counters window (ACQUIRE -> Counters).



You can control the speed (Events/Second) by adjusting the flow rate. The cytometer can handle 3000 events/second (we've gone as high as 5000 events/second without any problems). The flow rate will start at LO (default). You can increase the rate to MED or HI by pressing the flow rate buttons on the cytometer. Usually you will run setup on LO and acquisition on HI. Note that Total Events includes Gated and Ungated events, so it may get significantly higher than 10,000 before it's done.

Once your sample is done it will be saved (the histogram will turn purple). If it looks like there's no way you will approach the desired number of cells, you can press PAUSE and then SAVE on Acquisition Control to manually save your file.

When your sample is finished, take it off the SIP and put in the next one. Give it a new file name and repeat the acquisition procedure.

Data analysis:

This is a big topic and I will only give it very light coverage. Basically, you will use your CellQuest Pro document to do your analysis. Once your data is acquired, you can open new plots. These will be Analysis plots (instead of Acq->Analysis). Use the Inspector to select the desired file. Select the gate and axes. For histograms, you can overlay multiple data sets (select PLOTS -> Overlay) for comparison. Use the STATS menu to pull up statistical data about your samples. For example: choose Histogram Stats for a selected histogram plot. You will need to select your data set as both Numerator and Denominator. Press OK to display statistics like mean fluorescence values, etc. There is a lot you can do here.

This should get you through the simplest of one-color experiments. Once you've gotten the hang of it, it won't be too much trouble to learn two-colors and beyond.

Happy FACSing!

Note:

Here are the default Instrument settings:

```
Cytometer Type:  FACSCalibur

Detectors/Amps:
Param     Detector Voltage  AmpGain  Mode
P1        FSC      E00      1.00     Lin
P2        SSC      350      1.00     Lin
P3        FL1      600      1.00     Lin
P4        FL2      550      1.00     Lin
P5        FL3      650      1.00     Lin
P6        FL2-A              1.00     Lin
P7        FL2-W              1.00     Lin

Threshold:
Primary Parameter:  FSC
Value:  52

Secondary Parameter:  None

Compensation:
FL1 - 0.0 % FL2
FL2 - 0.0 % FL1
FL2 - 0.0 % FL3
FL3 - 0.0 % FL2
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